NEUROMUSCULAR PHYSIOLOGY OF A SESSILE SCYPHOZOAN

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Interest in the coelenterate neuromuscular system has persisted for many years, and information gained by a variety of approaches. Pantin (1935a, 1935b, 1935c, 1935d) first introduced the use of controlled electrical stimuli and the observation of subsequent muscular contractions as a means of analysis of the properties of the conducting system in Actiniaria. Bullock (1943) has shown that the propagation of the swimming pulse in Scyphozoa differs in no fundamental way from excitation in anemones, although there are certain quantitative differences that result in the remarkable dissimilarity of behavior of the two groups of organisms. In anemones, contraction of the muscles is relatively rapid, but relaxation is slow, and because of neuromuscular facilitation each impulse after the first arriving at the muscle excites further contraction so that a typical "staircase" is recorded from the contraction of the sphincter muscle in *Calliactis*. In scyphozoan medusae the first impulse is effective in eliciting a response. Each succeeding impulse, if delivered within a well defined time limit, enhances the response for several contractions until a plateau is reached which may be maintained for long periods of time. After each contraction the muscle relaxes completely (or almost so) so that the typical kymograph record appears as in Figure 1, a recording of *Cyanea* bell contractions. This is the result of rapid contraction, rapid relaxation, the restoring force of the mesoglea, and an extraordinarily long absolute refractory period (0.7 second, according to Bullock, 1943) of the muscles of the medusa bell.

As indicated above, all critical work using controlled electrical stimulation has been confined to Anthozoa and free-swimming jellyfish. This raises the question of the relationship of response to mode of life. Are the phenomena described sharply divided along systematic lines, or are there some definite and demonstrable properties that may be associated with the sessile habit or the free-swimming habit? The Stauromedusae, about which very little is known, offer ideal material for such a study.

Except for a brief creeping larval phase, Stauromedusae are sessile throughout their existence, but there is no doubt of their affinities with the Scyphozoa. Do they retain the neuromuscular patterns of their nearest relatives, or has the sessile habit led to modifications (or retentions from the scyphistoma larva) that make the Stauromedusae functionally more closely associated with the anemones? Although Stauromedusae lack the ability to perform pulsating swimming motions, one might wonder if the system is capable of such contractions under artificial stimulation. Is the absence of a pacemaker the only essential factor here?

The comparative aspect of the problem would be ideally developed by studying:

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(a) the responses of anemones; (b) the responses of free-swimming Scyphozoa; (c) the responses of the scyphistoma larva of the Scyphozoa; and (d) the responses of Stauromedusae. A knowledge of all of these might enable one to assess the consequences of the sessile habit and give some insight into the evolution of their neuromuscular mechanisms and behavior. The present study deals only with the last of these.

Materials and Methods

Haliclystus auricula (Rathke) was used because of its abundance near the Friday Harbor Laboratories of the University of Washington. Large individuals were collected, along with a portion of the blade of *Zostera* to which they are normally attached. The animals were maintained in the laboratory in running sea water at a temperature close to that of their natural environment (10–13° C.). Reactions were first explored by means of manually controlled mechanical stimuli and later by controlled condenser discharge shocks. Recordings were made with very light isotonic levers on a smoked drum.

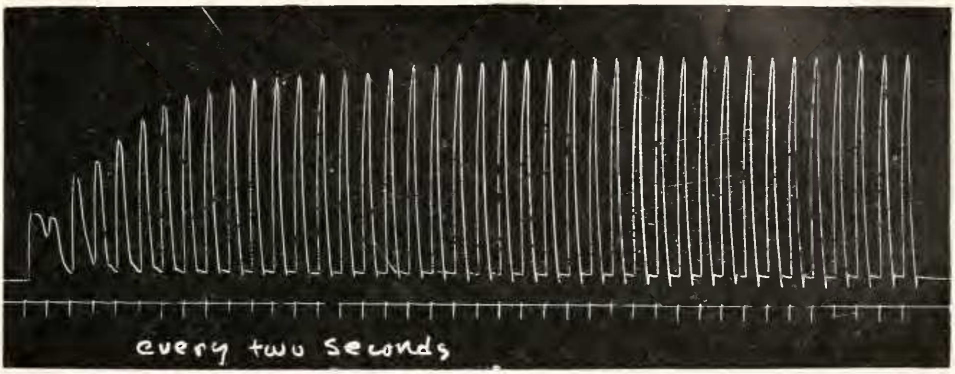

FIGURE 1. *Cyanea capillata.* Record of bell contractions. Electrical stimuli, 0.5 per second.

Electrical stimuli were delivered under sea water to the exumbrellar surface, usually at the base of the stalk or at the stalk-calyx junction, by means of silver electrodes insulated to the tip, with Ag-AgCl-sea water electrodes or non-polarizable calomel electrodes. A student "Electrodyne" stimulator, which delivers brief condenser discharge shocks, and for some experiments a Grass model S4B stimulator, were used. All experiments were made on fresh animals at temperatures of 11–13° C. At temperatures above 16–18° C. responses became very erratic, probably due to the rapid decay of facilitation (Hall and Pantin, 1937; Pantin and Vianna Dias, 1952). Mechanical stimuli were delivered with a clean, blunted glass rod or with a glass rod tipped with a short length of silver wire.

Anatomical studies to determine the details of the musculature were carried out by observation on entire living and preserved animals under reflected and transmitted light and between crossed polaroids. To confirm the results obtained from a study of entire animals, portions of several were sectioned and stained with picro-indigo-carmine following bulk staining in Grenacher's borax carmine, or with alum hematoxylin and eosin.

I would like to express my gratitude to the Director and Staff of the Friday Harbor Laboratories of the University of Washington for a grant which made residence at the Friday Harbor Laboratories possible for a period of seven months. My thanks are also due to the Department of Zoology, University of California, Berkeley, for support and encouragement over a period of several years. Dr. Ralph I. Smith and Dr. Cadet Hand have aided in many important ways, not the least of which was a critical reading of the manuscript. Their interest and criticism is deeply appreciated. Dr. T. H. Bullock has been kind enough to read the manuscript. His criticisms and suggestions have been very helpful and are gratefully acknowledged.

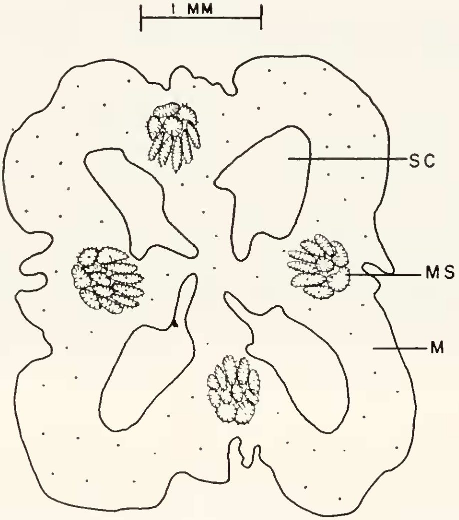

FIGURE 2. Cross-section through the stalk of *Haliclystus auricula*. SC, stalk canal; MS, stalk muscle; M, mesoglea.

RESULTS

Musculature of Haliclystus

It is possible to divide the muscles of *Haliclystus* into three categories associated with particular regions of the body. The stalk possesses four interradial muscular bundles (Fig. 2) embedded in the mesoglea which continue distally into the subumbrellar ectoderm as eight perradial sheets that fan out over the subumbrellar surface (Fig. 3). Proximally, the stalk muscles are inserted at the pedal disc over a rather wide area. There is also a marginal muscle (subumbrellar) which borders the margin of the calyx (Fig. 3) and is only partially embedded in the mesoglea. This latter muscle is interrupted at the arms and so consists of four perradial and four interradial segments. In addition, arm tentacles, gastric tentacles, manubrium, and anchors are provided with muscle fibers. The principal muscles involved in the reactions studied, however, are those of the stalk, subumbrella, and margin. With the possible exception of the stalk musculature, all the muscles of *Haliclystus* are confined to the subumbrellar surface, just as in free-swimming Scyphozoa. Thiel (1936) states that there are probably fine muscle fibrils distributed all over the body, although their existence has not been established, and no particular function has been ascribed to them. I have been unable to determine the presence of exumbrellar muscle fibers in *Haliclystus* and believe that all of the gross actions of the organism can be explained on the basis of interactions of the well-defined muscle fields and the mesoglea.

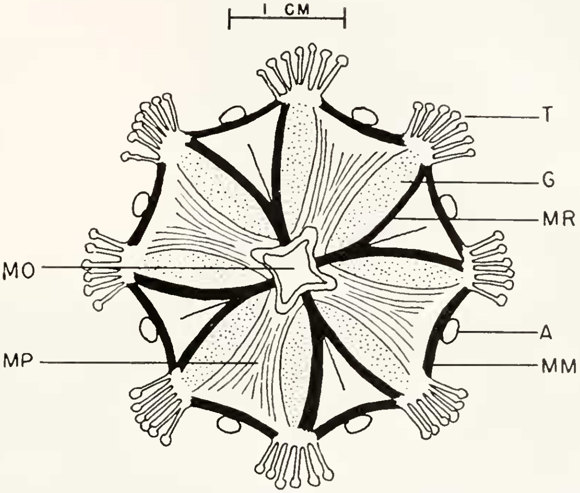

FIGURE 3. Diagram of *H. auricula* subumbrella to illustrate musculature. T, tentacle group; G, gonad; MR, radial muscle; A, anchor; MM, marginal muscle; MO, mouth; MP, perradial muscle.

Spontaneous activity

A considerable amount of apparently spontaneous activity may be observed in *Haliclystus* under both field and laboratory conditions. The arms are flicked in toward the mouth, the calyx is rotated, the stalk alone or the whole animal may contract without any noticeable external stimulation. No well-defined rhythm is apparent from visual observations, nor is any particular order of contracting parts consistently manifested.

Kymograph records of this activity were made on slow drums. There is no orderly progression of height of contraction as in free-swimming scyphozoan jellyfish. The contractions recorded are due primarily to the stalk muscles and do not show the contractions of individual arms. However, such recordings serve to illustrate the arrhythmic nature of spontaneous activity of unmolested animals (Fig. 4, A).

It was apparent from the outset that difficulty would be encountered in attempts to record responses to electrical stimuli in such an active animal. With this in mind, various parts of the body were isolated, to ascertain whether or not any structure or structures exercised a "pacemaker" control over any other part. Considering the systematic position of the animal, the most obvious place to look for such control was the umbrellar margin, which, in free-swimming scyphomedusae, bears the rhopalia, known to be the seat of pacemaker control of the swimming pulsations. In the majority of medusae (both Hydrozoa and Scyphozoa) removal of the bell margin results in a more or less complete paralysis of the swimming movements (Romanes, 1885; Bullock, 1943). No such pacemaker region has been demonstrated in anemones or in hydrozoan polyps, but in the case of anemones, the spontaneous activity is of such a slow nature as to allow investigation of neuromuscular responses with considerable confidence. In *Haliclystus* this activity occurs with sufficient frequency to be extremely troublesome.

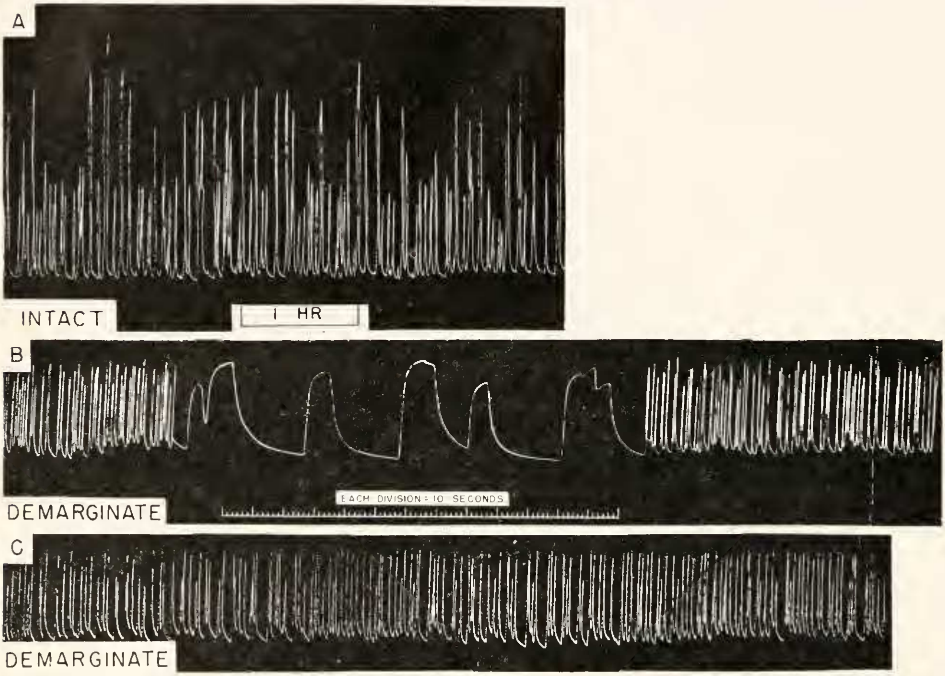

FIGURE 4. Records of spontaneous activity. A. Intact animal. B. Stalk only. Drum speed increased in central portion for 15 minutes. C. Stalk only. Hour marker applies to entire record except central part of B.

In order to determine whether or not "surgical paralysis" was possible, a large *Haliclystus* was set up in such a manner that stalk and calyx contractions could be recorded independently and simultaneously. Pins were placed at the stalk-calyx junction, fastening the animal to a wax-bottomed dish so that the two parts could be separated without disturbing the mechanical recording devices. Contractions of the intact animal were recorded for several hours, after which a sharp knife was drawn across the animal at the stalk-calyx junction, isolating each part from the other, and the recording continued for several more hours. Examination of the record shows that no difference can be observed in the nature of the contractions before and after separation. Further recordings were made of intact animals and isolated stalks, and no difference was noted (Fig. 4).

Removal of arm tips (tentacles), anchors, bell margin, and, as has been seen, the whole calyx, has no effect on the nature of spontaneous activity. From the above, it is apparent that there is no physiological centralization of the exciting system that manifests any pacemaker control over any other part. The mechanism of this type of activity has not been established directly, but may be inferred from evidence to be presented later.

Responses to mechanical stimulation

The stalk and exumbrella: Stimulation of the surface of the stalk and of the exumbrella in attached and unattached *Haliclystus* seldom elicited a response. Gentle stroking of the exumbrellar surface, stimulation by light touch, and fairly vigorous prodding almost invariably failed to call forth a response. If, however, firm pressure were maintained with the glass rod, *e.g.* on the edge of the pedal disc, a sudden shortening of the stalk and closure of the bell resulted. On several occasions the response was elicited only upon injury to the animal, and continued pressure did not cause a maintained calyx contraction. Shortly after the sudden closure the animal would relax, the calyx relaxing first, even if pressure sufficient to cause injury were maintained. This response could be obtained from any point on the exumbrellar surface and did not appear to be restricted to the pedal disc. The pedal disc does not appear to be any more sensitive than other areas, although the methods used would not give information on any slight gradient of sensitivity. After numerous attempts at mechanical stimulation of the exumbrella, it was concluded that the exumbrellar surface was very nearly insensitive to ordinary mechanical stimulation, responding only to very vigorous stimuli.

This is not surprising when one considers the conditions under which *Haliclystus* normally lives. The population from which experimental animals were taken is almost completely confined to rather dense beds of *Zostera marina*, with the animals usually attached to the blades in a pendant attitude. The *Zostera* is exposed only at the lowest tides, which means that for the greater part of the life-cycle of the animal it is hanging in the water among constantly moving blades of the plant, and is therefore subjected to a considerable amount of buffeting. The exumbrella would be exposed to repeated stimulation by blades of *Zostera*, debris in the surrounding water, and perhaps fairly frequent rubbing over the bottom. If these medusae were very sensitive to exumbrellar stimulation, they would be in the contracted state for a major part of their existence.

Subumbrellar surface and associated structures: The tentacles are extremely sensitive to weak mechanical stimulation. The slightest touch to a single tentacle usually brings about an arm-bending response. Sometimes only a few tentacles react, but more often there is a rapid bending of the whole arm toward the mouth. The arm tips at the base of the tentacles on the exumbrellar side are quite insensitive if the stimulus is applied outside the area of the tentacles, but are quite sensitive on the subumbrellar side.

The entire subumbrella is very sensitive to mechanical stimulation. If the stimulus is applied on the adradius, the arm in the position responds by bending toward the stimulated spot, and frequently the arm on either side also responds. If the stimulus is applied between the two arms (*i.e.*, in the inter- or perradius), the two adjacent arms respond by bending to the stimulated spot, and frequently, the next adjacent arm on either side also bends. In addition it was noted that the manubrium moved toward the stimulated area whenever the arm-bending response was obtained.

Repeated stimulation of a single spot on the subumbrella may either bring about a total calyx closure, or local insensitivity develops and the animal fails to respond. If a slight stroking movement covering several millimeters is made with a glass rod or silver wire, the total closure response results. It was noted on several occasions that repeated light touches in one spot would bring about total calyx closure in a fairly regular fashion. First the adjacent arms would bend toward the stimulated spot, then on the next stimulus the next adjacent pair, and so on until all eight arms were bent inwards. The exact relationship between stimulus and response in such cases was difficult to assess. If the stimulating object were not removed quickly, it would be caught by the tentacles and the stimulus of this contact would almost invariably cause total calyx closure. Quick removal of the glass rod, however, may have caused sufficient local disturbance to provide additional sources of stimuli, so that accurate appraisal of the stimulus-response relationship under these conditions seems impossible.

If the total calyx closure response were elicited by the stimulus of a linear stroke, stalk contraction and a characteristic rotation of the calyx usually occurred as well. The calyx rotation appeared simultaneously with the stalk contraction.

It was noted that any adequate mechanical stimulus delivered at the edge of the pedal disc always brought about a calyx closure reaction in addition to the stalk contraction, but that adequate calyx (subumbrellar) stimulation did not always elicit stalk contraction. This might be considered evidence of polarization in the conducting system, in the sense that it is "easier" to drive an impulse in one direction than in the other, but due to the methods of stimulation and the extreme difference in the intensity of "adequate" stimulation of the two areas concerned, it would seem unwarranted to accept this observation as evidence of polarity.

Conduction of excitation may be described as diffuse. A single touch elicits the arm-bending response in two to four arms, as well as a shift of the manubrium toward the stimulated point. The so-called "decremental" nature of conduction is also noted in the spread of contraction around the calyx from repeated or more vigorous stimulation. That conduction of excitation is rather slow is quite evident from observations made during mechanical stimulation. The arm nearest the site of stimulation always responds first, the other two following in order depending on whether the stimulated point is nearer one than the other. If they are equidistant they react simultaneously. The reaction is sufficiently slow so that the above is readily observed. Thus, the special properties of the nerve net as outlined by Pantin (1935a) are apparently fulfilled in *Haliclystus*.

Response to electrical stimulation

It has been indicated previously that the normal activity of *H. auricula* presents an unpredictable variable when one attempts to determine the responses of the organism to artificial stimulation. I found no convenient method of suppressing this activity. Further difficulty was encountered in determining the area to be stimulated. The demonstrated insensitivity of the exumbrellar surface offered the advantage that placement of the electrodes on it caused little disturbance, but this insensitivity may have been the result of absence of nervous elements in the exumbrellar ectoderm. If the electrodes were placed on the subumbrellar surface there was an immediate response to the tactile stimulation, and after a time local insensitivity developed.

The site finally selected was the exumbrella. Kassianow (1901) demonstrated what appears to be a nerve plexus in the exumbrellar ectoderm of Stauromedusae, and experiments to be described support this. Insensitivity to mechanical stimulation may be due to rapid sensory adaptation or to high sensory thresholds and paucity of mechano-receptors in the exumbrella. Neither of these possibilities has been directly demonstrated, but the responses suggest such explanations.

It was necessary to exercise caution in setting up the animals for recording. When possible, the animals were left attached to the *Zostera* on which they were collected and the plant pinned to the bottom of a wax- or clay-lined container. Small glass hooks were used to attach the animal to the recording lever. These were inserted through the bell margin beneath the marginal muscle at one side of an anchor. If the hooks were placed through the arm tips the animals remained unduly agitated for some time, presumably due to stimulation of the tentacles. Each preparation had to be observed carefully to eliminate the possibility of additional stimulation from the recording connections. Animals were not used until three to four hours after having been set up, to allow recovery from the effects of injury. Tests indicated that the responses did not differ appreciably after one-half hour from those after twenty-four hours following injury. All recordings were made from animals in standing sea water.

Response to single-impulse stimulation: As indicated previously, a single condenser-discharge shock results in a contraction of small amplitude in free-swimming Scyphozoa (Bullock, 1943). In the anemones that have been studied (Pantin, 1935a; Hall and Pantin, 1937; Pantin and Vianna Dias, 1952), single-shock stimulation does not result in a recorded response. To determine the response of *Haliclystus* to this type of stimulation, a large specimen was set up for recording and the electrodes applied to the stalk ectoderm.

As might be expected, the results were variable. Stimulation was being applied against a background of spontaneous activity. Contractions were recorded during the course of these experiments, but they were not always associated with the stimulus. Responses occurred before, after, and at stimulation in an apparently random manner. There was no consistent contraction associated with the delivery of the stimulus until high intensities were reached. Examination of preparations receiving such shocks revealed an injured area at the site of the electrodes, and these responses were interpreted as a result of multiple stimuli resulting from injury. Because of the fact that contractions occurred at random with this type of stimulation except at high voltages, it was concluded that *Haliclystus* does not characteristically respond to single-impulse exumbrellar stimulation.

The failure to respond to single-impulse stimulation also characterizes reflex closure of the sphincter of the anemone *Calliactis* (Pantin, 1935a) and the response of the longitudinal mesenteric muscles of *Metridium* (Hall and Pantin, 1937).

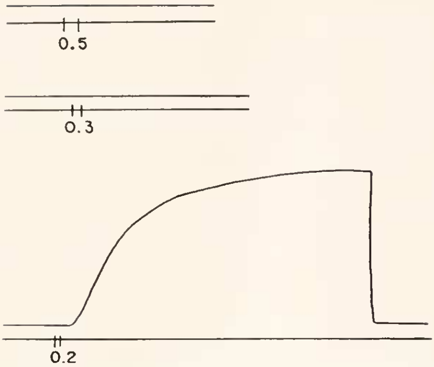

FIGURE 5. Tracings of records of stalk response to pairs of shocks. Upper trace of each pair, contraction record; lower trace, signal marker. Interval between shocks in seconds.

Following single-shock stimulation in *Haliclystus*, a twitching of the tentacles was often observed, as has been reported for *Calliactis* by Pantin (1935a), who considered it an indication that the impulse was being transmitted. In scyphozoan medusae there is a consistent measurable response to a single stimulus, although the magnitude of the response is very much less than that of which the preparation is capable (Bullock, 1943).

Response to pairs of stimuli: After having established the failure of *Haliclystus* to respond in a consistent manner to single-impulse stimulation, the response to pairs of stimuli was investigated. Pairs of shocks at a frequency of one in two seconds, and one, two, three, and five per second were delivered to the preparation. A standard five-minute rest period was observed between the delivery of each pair of stimuli, but because of spontaneous activity this interval was frequently extended. At least one minute of observed inactivity was allowed before the delivery of stimuli. A record of a typical experiment of this sort is shown in Figure 5. It will be noted that a response does not occur until the members of the pair of stimuli are separated by only 0.2 second, *i.e.*, at a frequency of five per second. Occasionally a response occurred at three per second, but the illustrated case is more typical.

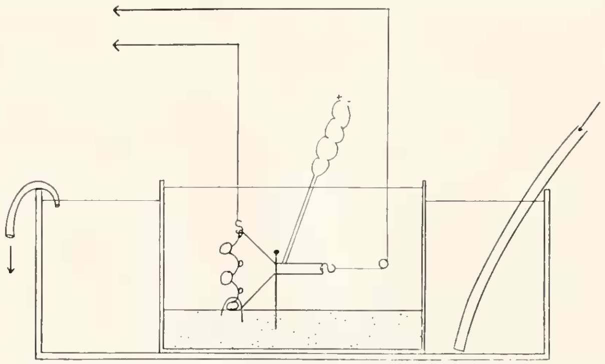

FIGURE 6. Diagram of experimental set-up for simultaneous stalk-calyx recordings.

During the course of the investigation described above, it was noted that total calyx closure regularly occurred at a lower frequency stimulation than stalk contraction. Because of the recording method, such calyx contractions were not recorded. Accordingly, animals were set up in such a way that independent and simultaneous records could be obtained of stalk and calyx contractions (Fig. 6). Stimuli were delivered on the exumbrellar surface at the stalk-calyx junction in the same manner as in the previous experiments. These were repeated on individuals without the encumbrance of the recording and holding devices, and in all cases the results corresponded to those shown in Figure 7. The calyx contracts weakly at a frequency of two per second, more strongly at three and five per second; at the latter interval the stalk contracts, as would be predicted from the previous determination. In contrast to the observed reactions to single stimuli, the responses to pairs of stimuli under the conditions described above are remarkably consistent.

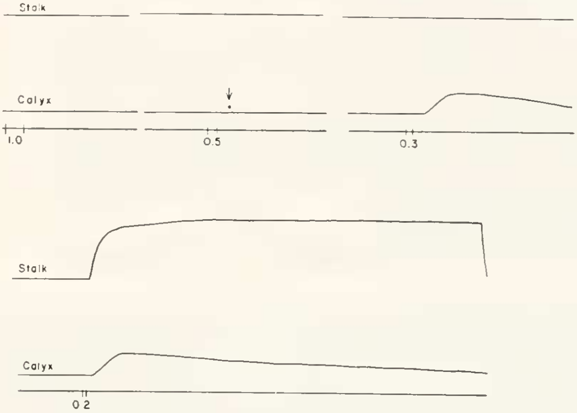

FIGURE 7. Tracings of records obtained as illustrated in Figure 6. Lower trace, signal marker. Interval between shocks in seconds. Unrecorded contraction observed at arrow.

Characteristic response frequencies for different parts of the responding system have been demonstrated in anemones by Pantin (1935b) and can be related to the function of the parts. In *Haliclystus* the calyx is the feeding organ of the animal and as such must be sensitive to very light mechanical disturbances if it is to fulfill its function.

Responses to trains of stimuli: The response to pairs of stimuli is essentially a total response. It is evident on observation of unmolested animals that natural responses are graded, *i.e.*, by no means all responses to naturally occurring stimuli involve all of the responding muscles to the full extent. It is also apparent that each of the three categories of muscles described earlier is capable of at least some independent action. The calyx is able to contract (as has been shown) without stalk contraction, and the stalk under certain conditions may show slow, partial, and frequently asymmetrical responses without the calyx being involved. This is apparent in the characteristic movements by which the expanded calyx is swept through the water, rotated, and inclined at various angles. The marginal muscle is also apparently capable of independent activity, as indicated by certain postures

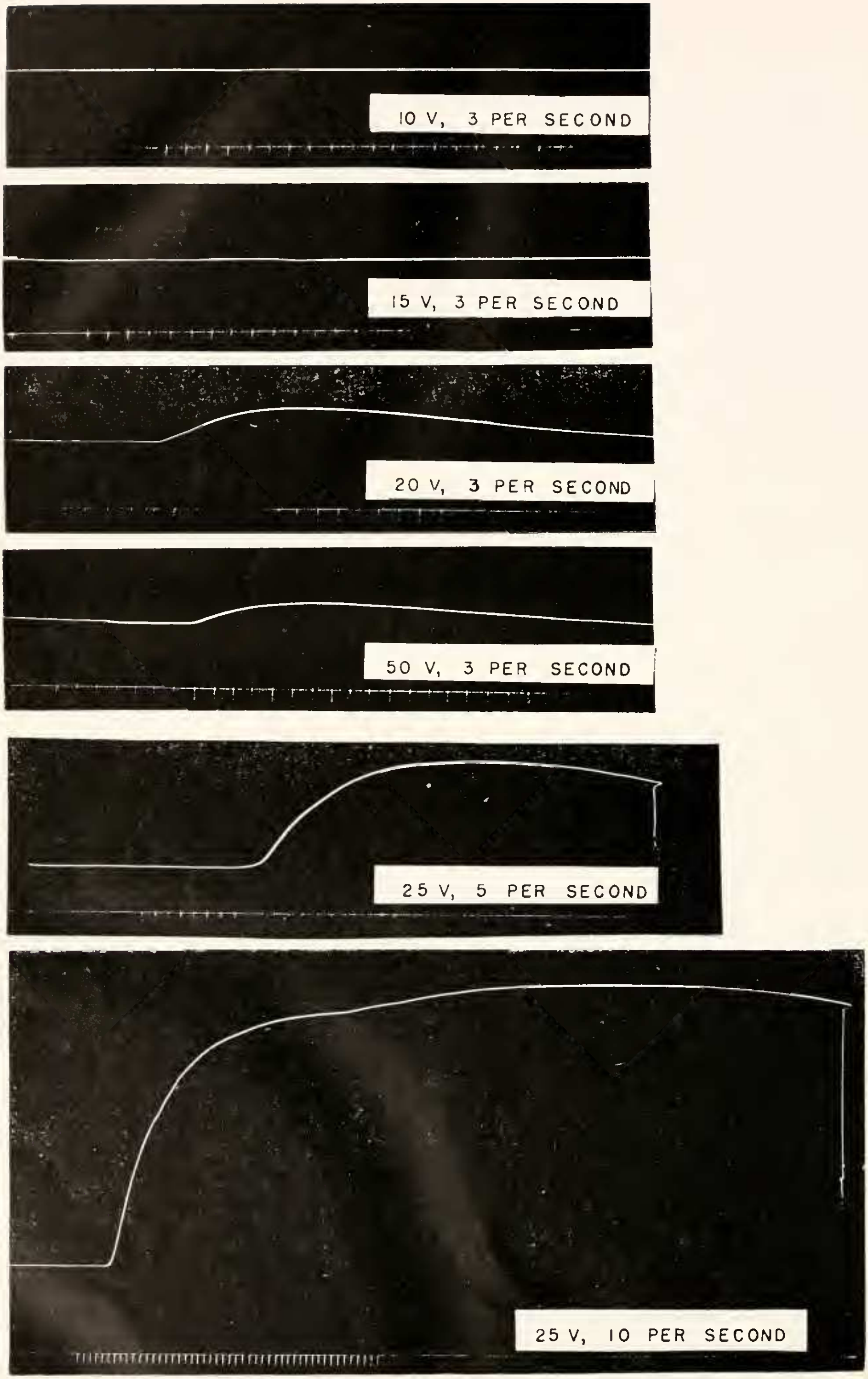

Figure 8. Records of stalk responses to various voltages and frequencies of electrical stimulation.

the animal assumes. In the expanded individual it is noted that the eight adradial arms are most frequently equidistantly arranged. At times, however, the arms are drawn together so that they are associated in four pairs, and such pairs may lie in the perradii or, more frequently, in the interradii. Examination of such animals reveals slight folding of the mesogiea over the marginal muscle between the pairs of arms and the corresponding radial muscles of the subumbrella. The intervening muscles show no sign of contraction.

In order to investigate the underlying mechanism of the graded response, the reactions to a variety of stimuli were examined. No information concerning the mechanism of the postural response has been obtained, but certain inferences concerning symmetrical graded responses may be made from reactions to trains of stimuli at different frequencies. Because of the spontaneous activity of the animals, it was impossible to interpret the results when long trains of stimuli at very low frequencies were administered. Any observed contraction could just as well be spontaneous as a reaction to the applied stimuli. At intermediate frequencies, however, *i.e.*, lower than that required for a response to a single pair, but higher than one shock every two seconds, a consistent pattern emerged.

Figure 8 illustrates a possible mechanism of graded response. It will be noted that there is a well-marked threshold and that increasing the intensity of the stimulation does not appreciably affect the height of contraction. At voltages just above threshold, however, increased frequency of stimulation has a marked effect on contraction amplitude.

A similar experiment was conducted on an animal set up for simultaneous stalk and calyx recording, as in Figure 6. The results shown in Figure 9 were recorded from the same animal in the space of less than one-half hour. It will be noted that there is no evidence of a well-marked "staircase" in any of these responses, as has been observed in the anemone *Calliactis* by Pantin (1935a), but that the contractions are essentially continuous. This may be interpreted as the result of the relationship between speed of contraction and decay of facilitation if factors similar to those in other coelenterates are operative. There is none of the machine-like precision in *Haliclystus* that is demonstrated by anemones and free-swimming Scyphozoa. This is probably because of the complicating factors of frequent spontaneous activity, speed of contraction, and the rapid decay of facilitation.

Responses to subumbrellar stimulation: The responses to electrical stimuli reported above are from stimulation of the exumbrellar surface, and are essentially only symmetrical responses. Asymmetrical responses are clearly possible, as indicated by mechanical stimulation and by observation of the undisturbed animal. Certain of these reactions may be examined by subumbrellar electrical stimulation despite the disadvantages pointed out earlier. Such responses have not been recorded because of the weakness of the muscles involved. In some cases, isolated calyces were used, while in others the observations were made on intact animals.

Upon placement of the electrodes there was an immediate and sometimes repeated response, either asymmetrical or total in nature. Electrodes were placed as lightly as possible, but the response ensued upon contact, however light. The animal was then left undisturbed for at least fifteen minutes.

Because of the extreme sensitivity of the subumbrella, precise analysis of the neuromuscular properties was very difficult. Single-impulse stimulation frequently elicited a localized response involving only one or two arms, as was observed following light mechanical stimulation. Single-impulse stimulation was never observed to cause a generalized response, but pairs of stimuli at 0.5- to 0.1-second intervals would cause a generalized contraction. Pairs at greater intervals would cause either two localized contractions coincident with stimulus delivery or would spread to the adjacent arms, depending on the interval. If several stimuli were delivered at a frequency of one per second, the contraction would spread in both directions around the calyx, bringing successive arms into contraction coincident with each successive stimulus. Those already contracted would contract again. Thus, the total calyx response could be elicited in a stepwise manner.

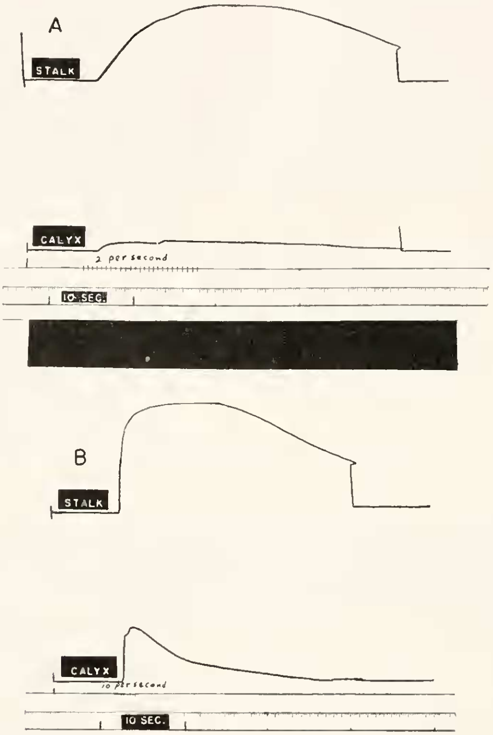

FIGURE 9. Simultaneous stalk-calyx contractions recorded as for Figure 7. Contraction records inked to increase contrast. A. Response to a train of shocks at 2/second. B. Response to a train of shocks at 10/second. Each preparation received 25 shocks.

A possible explanation of these results is that there is an immediate spread of the impulse to the neuromuscular junctions on the first stimulus and then neuromuscular facilitation governs the excitation of the muscle. A single pair of stimuli at a sufficiently short interval excites the whole system, but it takes several stimuli at lower frequencies to accomplish this. This is probably related to the rapid decay of facilitation, and suggests through-conducting tracts in the subumbrella which, on the basis of this evidence, might be syncytial. Evidence to be presented later, however, suggests that there is interneural facilitation as well.

Conduction speed in the "nerve net"

A number of attempts to measure speed of conduction in the system were carried out. Each determination was repeated several times before any consistency was obtained, and even then there was considerable variation. A strip of tissue was cut from the calyx, leaving one end of this tongue attached to the animal, which was then pinned to a wax-bottomed dish with the stalk projecting upwards. The stalk was connected to a recording lever in the usual way. A pair of stimuli was delivered at an appropriate interval distally on the tongue of tissue, and after five to ten minutes' rest, a similar pair was delivered at a measured distance proximal to the original site. The time from the second stimulus to contraction was carefully measured for each pair of stimuli, and the speed of conduction calculated from the difference in time and the distance between the stimulating electrodes. This was done on several animals, and the values obtained were 7–15 cm./sec. at 11–12° C., comparable to those of 10–20 cm./sec. obtained for *Calliactis* body wall by Pantin (1935b).

Other attempts were made by placing the electrodes at two different points on a line with the reacting muscles on the exumbrella in the intact animal. The results of such determinations varied so radically that they have not been included. Originally the assumption was made in these experiments that no conducting tissue crossed the mesoglea, and that the route of the impulse from stalk stimulation was up the stalk ectoderm, over the bell margin and down the subumbrella to the stalk muscles. The results suggested that there was transmission through the mesoglea at some point along the route.

The form of the "nerve net"

The most careful study of the nervous system of the Stauromedusae is that of Kassianow (1901). In a very thorough investigation using various staining techniques, including vital methylene blue, Kassianow studied the histology and cytology of the nervous system and associated structures in *Lucernaria* (= *Calvadosia*) *campanulata*, *Haliclystus octoradiatus* (= *H. auricula?*), and *Craterlophus tethys*. He describes and figures a diffuse exumbrellar plexus of bi- and tripolar ganglion cells. The experiments to be described largely confirm Kassianow's morphological findings.

Exumbrellar transmission of excitation: In order to test for the presence of exumbrellar nervous elements, several large specimens of *Haliclystus auricula* were selected and a circular incision was made through the stalk ectoderm at a level four to six millimeters above the pedal disc (Fig. 10, A). Following the operation the animals were given two to four hours in which to recover and were then stimulated on the stalk ectoderm aboral to the cut. Under these conditions a contraction could not be elicited.

Stimulus intensities up to 100 volts failed to cause excitation at frequencies at and above threshold for normal animals. Simply transferring the electrodes to the oral side of the cut resulted in excitation at normal frequencies and voltages. Animals were examined for muscle and endodermal continuity before and after stimulation. All animals showed continuity, although there was some damage to stalk canals in all specimens except one which gave the same results as the others.

The stalk of another individual was split from the base up toward the calyx and a cut made from the inside out toward the ectoderm, leaving only the ectoderm in continuity, and stimuli applied basal to the cut (Fig. 10, B). Under these conditions excitation occurred at normal voltages and frequencies. Subsequent examination revealed that a strip of ectoderm two to three millimeters wide was the only continuous tissue except for the very superficial mesoglea underlying the strip. This indicates that conduction of excitation is diffuse. If the conducting tissue is in the form of fibers, these are probably diffuse; but if conduction is a general property of the ectoderm or other superficial layers, one would also expect the results obtained. The latter possibility seems unlikely, but is not excluded by the evidence.

It might then be assumed that the normal path of exumbrellar transmission is over the exumbrellar surface to the bell margin, over the marginal edge to the subumbrellar muscles and to the stalk muscles. To test this assumption, the entire subumbrellar surface, including the oral region, was removed from an animal (Fig. 10, C). The ring muscle was left intact, as were the distal ends of the gonads, the arm tips, and tentacles. Under these conditions there is no ectodermal continuity from any point on the exumbrella to the stalk muscles, and since the radial muscles of the calyx have also been removed, transmission cannot take place through them. Stimuli were then delivered at the base of the stalk, and at normal voltages and frequencies a response of the stalk muscles was elicited. Such a preparation does not respond to a pair of stimuli at 0.2-second interval as does an intact animal, which may indicate that the normal pathway of excitation has been interrupted. Also, this preparation still had some septal tissue, which may have given continuity.

To insure no marginal continuity, another animal had the entire upper portion of the calyx removed as well as the subumbrellar tissue (Fig. 10, D). Under these conditions, a contraction could also be obtained at normal voltages and frequencies, although it took four shocks at five per second to elicit the response.

Still another animal was completely demarginated, the stalk split from the base up to within one millimeter of the cut edge, and stimuli applied at the base of one-half of the stalk (Fig. 10, E). This meant that if excitation were transmitted to the other half, it would be forced to traverse the mesoglea at some point in the non-stimulated half after having gone through the one-millimeter strip of ectoderm at the cut margin. The four groups of stalk muscles are separated by mesoglea at this level, and there is no opportunity for transmission through the split base of the stalk. Under these conditions, the non-stimulated half responds to shocks at normal voltages and frequencies. In this preparation there was some continuous endodermal tissue at the oral end, but the previous demonstration of the necessity of ectodermal continuity for transmission in the stalk reduces the significance of this. Furthermore, it does not affect the contention of mesogleal transmission, because neither the endoderm nor the ectoderm is in contact with the muscles.

Wietrzykowski (1912) clearly shows the development of the stalk muscles from the "cordon cellulaire de la taeniole" which has its point of proliferation in a subumbrellar position. This means that the direct innervation of the stalk musculature from the exumbrellar ectoderm is a result of conducting tissue arising in the exumbrellar ectoderm and becoming associated with the musculature, or of con-

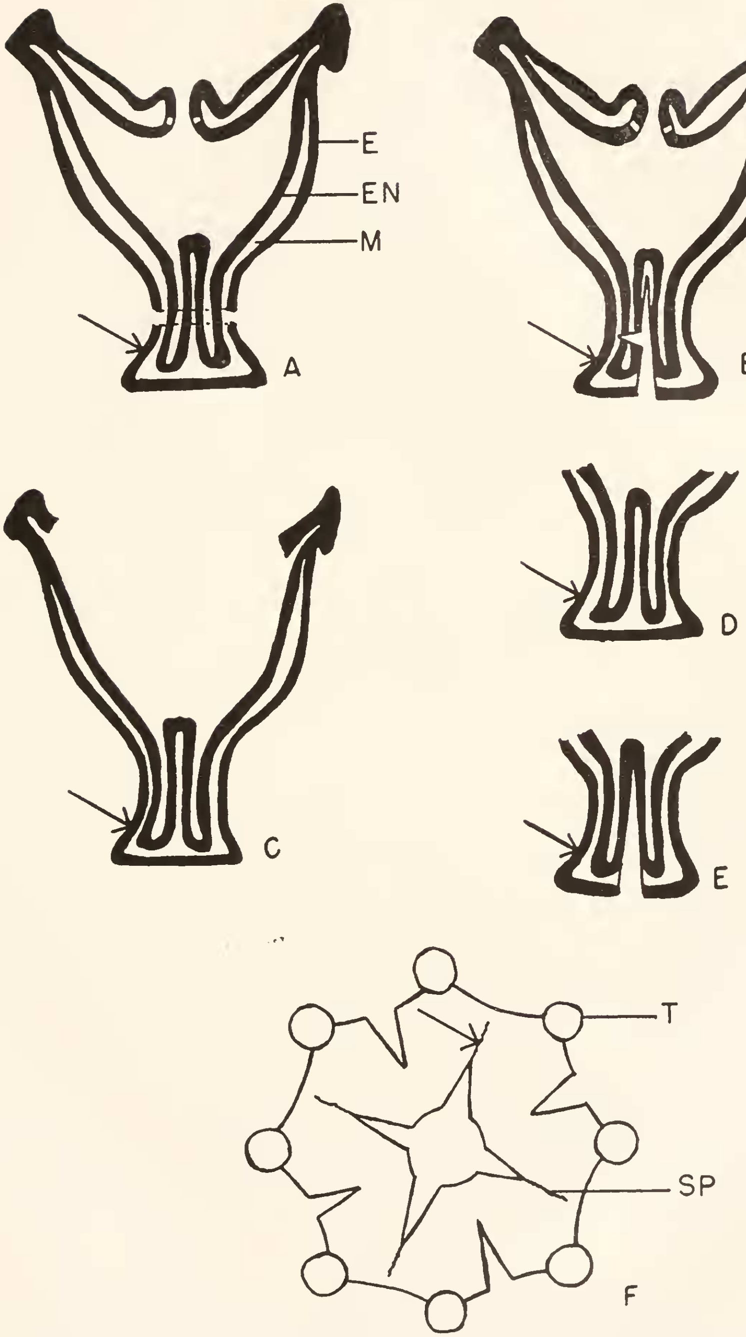

FIGURE 10. Diagrams to illustrate operations to ascertain the form of the conducting system. See text for explanation. E, ectoderm; EN, endoderm; M, mesoglea; SP, septum; T, tentacles. Position of stimulating electrodes indicated by arrows.

ducting tissue migrating out from the muscle to the ectoderm. Horridge (1956), however, has demonstrated a diffuse nerve net on the exumbrellar surface of the ephyra of *Aurellia aurita*, and it is possible that the exumbrellar conducting system of *H. auricula* is the homologue of the diffuse system in other scyphozoans.

Subumbrellar transmission of excitation: To ascertain the nature of subumbrellar conduction, the stalk and oral structures were removed from a specimen of *Haliclystus*. Four incomplete cuts were made from the margin to within a few millimeters of the cut inner surface between alternate pairs of arms, and similar cuts were made from the inside out toward the margin, alternating with the first cuts described (Fig. 10, F). This gave a ring preparation similar to those used by Romanes (1885) in which the impulse is forced to take a devious path to circle the bell. The electrodes were applied in the position indicated and trains of impulses were delivered to the preparation.

Under these conditions a train of impulses at normal voltage and at a frequency of five per second elicited a local contraction involving only the nearest two or four arms. At ten per second, the whole calyx could be brought into contraction in the following particular manner. First the arms nearest the electrodes respond, then those on each side in sequence and by pairs until all contract. There is a noticeable delay between the contractions of successive parts, as if junctions were being facilitated in turn. This preparation emphasizes the functional autonomy noticed in the intact animal. It also establishes the diffuseness of conduction and proves that no one pathway is essential for conduction around the bell. The expected pathway would be around the bell margin in the region of the marginal muscle, but the preparation described above shows that continuity of the muscle, and hence of any accompanying nerve tracts, is not essential to conduction. With arms and anchors removed, the preparation reacts in the same way as when these structures are present.

The above indicates that the arms are functionally associated into pairs. This functional pairing appears to depend on the integrity of the intervening marginal muscle and may indicate a normal through-conducting mechanism around the bell from arm to arm. Furthermore, if the stimulus is applied in the interradius, the contraction progresses by interradial pairs of arms. If applied in the perradius, the first reaction involves the adjacent arms only, or may involve the interradial *pair* on either side. This indicates that an interradial pair of arms, with its associated radial musculature (see description of musculature) and marginal muscle, has a degree of autonomy as a pair that separates it functionally from the other interradial pairs.

If the above results are examined in the light of stimulation of the intact calyx, the evidence for a through-conducting system is supported. That it is probably associated with the marginal muscle is indicated by the failure to respond to a pair of stimuli when this muscle is cut. It appears that under these conditions of mutilation, several junctions must be facilitated in order to bring about a generalized contraction.

Discussion

The results of this study enable one to construct a coherent picture of the normal behavior and responses of *Haliclystus auricula*. The pulsating contractions of the free-swimming Scyphozoa are lacking in *Haliclystus*. It has been demonstrated that there is no pacemaker present, and also that the requisite degree of specialization of the conducting system is lacking. Thus, the calyx of *Haliclystus* lacks the capability of responding in the repetitive fashion of its free-swimming relatives to repeated shocks at regular intervals. However, the available evidence indicates that a through-conducting system is normally operative in the subumbrella of the calyx. No evidence for higher conduction speed in that part of the animal, as compared to other parts, has been obtained. The evidence is primarily the different responses of experimentally incised and intact animals. The through-conducting system is brought into play only by relatively high frequency stimulation. At low frequencies the radial parts of the calyx exhibit considerable autonomy. In addition to this through-conducting system there also appears to be a diffuse "nerve net" which operates to correlate the subumbrellar structures, such as the manubrium, with the action of the principal food-gathering devices, the arms and the tentacles. There is no clear evidence of more than one nerve net as has been reported in *Aurellia* by Horridge (1956).

Inherent spontaneous activity may be interpreted in the following way: no part of the animal exercises control over any other part. From this we may assume that impulses are spontaneously generated in many, if not all, ganglion cells. Such impulses spread over the entire nerve net, but do not activate the muscles concerned unless two or more arrive at any one junction within an appropriate time interval. The nature of the spontaneous activity seems to indicate that each ganglion cell discharges at its own rate, each independently of the others. Activity may or may not be rhythmical. The graded and asymmetrical nature of the spontaneous activity, together with the lack of a regular pattern supports such an explanation. A pattern of endogenous activation of the muscles could lead to the observed sweeping, "seeking" motions of the unstimulated animal. Exogenous stimuli impinging upon the exumbrella must be of sufficient intensity to generate relatively high-frequency impulses if the protective closure-contraction response is to ensue, as indicated by the relatively high frequency of electrical stimulation required to bring about a response. The subumbrella, on the other hand, responds as a whole to lower frequency stimulation in a local fashion. Thus, if a food organism brushes very lightly over the subumbrellar surface a suitable reaction will be forthcoming in the arm-bending response to secure the prey. If the prey struggles, further impulses are generated, and the entire calyx responds to ensure capture and ingestion. The role of chemical stimuli in the predation process has not been investigated, but is no doubt of some importance—especially in the discharge of nematocysts and gland cell secretions.

If, as has been assumed, *Haliclystus* possesses a simple plexiform nerve net, the reactions reported in this study are subject to two possible interpretations. The high degree of autonomy noted in different regions of the subumbrellar structures is most easily explained on the basis of neuroneural facilitation at synapses between strategic areas of the net, or a hierarchy of thresholds of neuromuscular junctions to frequency and number of arriving impulses. Such a system, along with the endogenous activation of muscles, is admirably adapted to sweeping through the water in a "seeking" motion, and to catching prey on contact.

The exumbrellar nerve net shows a high degree of facilitation with rapid decay characteristics not noted in either anemones or free-swimming Scyphozoa, and this occurs at comparatively low temperatures (11–13° C.). The relationship of decay of facilitation (rapid) to contraction time (comparatively rapid) to relaxation time (relatively slow) is such that a fused contraction results at frequencies sufficiently high to elicit a contraction.

The subumbrellar nerve net exhibits the properties of through-conduction, neuromuscular facilitation and, probably, interneural facilitation. It responds locally to single-impulse stimulation, indicating a partial permanently facilitated state.

The properties of the neuromuscular system of *Haliclystus* do not appear to differ in any fundamental way from those noted in anemones (Pantin, 1935a, 1935d) and free-swimming Scyphozoa (Bullock, 1943). Modifications of the characteristics of the nerve net with regard to the time sequence of decay of facilitation and certain time characteristics of the muscles would appear to be sufficient to explain the differences in behavior noted in these animals. No new properties need be postulated to account for the reactions that do not fall in the range of variation already noted in the coelenterates.

Bearing in mind the presumed derivation of the Stauromedusae from free-swimming Scyphozoa, we may speculate upon the differences involved with the sessile habit. The most obvious of these is the loss of the pacemaker mechanism of swimming, and the loss of (or failure to develop) the specialized through-conducting mechanism that makes the swimming pulsations possible. Secondly, the stalk muscles are able to maintain a prolonged contraction not possible in free-swimming Scyphozoa. This is not as pronounced in the calyx musculature, which relaxes rather quickly even under sustained stimulation (see Figure 9). The properties of the mesoglea indicate that contraction is maintained against a mesogleal restoring force, hence the muscle must possess a refractory period of shorter duration than that found in free-swimming forms. Since sustained contraction differs in the stalk and calyx muscle systems, it is probable that each system has a muscle refractory period different from the other. Neither system is able to maintain a contraction for the length of time noted in anemones, so it may be reasoned that the physiological properties of both systems lie somewhere between those of free-swimming scyphozoans and those of anemones.

SUMMARY

1. Previous work on the neuromuscular systems of coelenterates has been confined to Anthozoa and free-swimming medusae, and certain differences in the response to electrical stimulation have been noted. It was thus of interest to investigate the responses of a sessile scyphozoan, *Haliclystus auricula* (Rathke), to determine the characteristics of the response mechanism.

2. Spontaneous activity of *H. auricula* is arrhythmic, and there is no demonstrable "pacemaker." The exumbrellar surface of the organism is relatively insensitive to mechanical stimuli, but the tentacles and subumbrellar surfaces are very sensitive. The conducting system possesses properties of a "nerve net." Conduction is slow, diffuse, and with an apparent decrement.

3. *H. auricula* does not respond to single-impulse electrical stimulation of the exumbrella. A pair of stimuli elicits stalk contraction at an interval of 0.2 second, and calyx contraction at 0.5 second. On the other hand, single-impulse stimulation of the subumbrella will elicit a response, but this response is local, involving only a part of the calyx near the position of the electrodes. A possible mechanism of the graded response is suggested.

4. Physiological evidence indicates that the conducting system is diffuse, with some evidence of a through-conducting pathway at the bell margin that operates at relatively high-frequency stimulation. Conduction speed in the subumbrellar nerve net is calculated at 7–15 cm. per second at 11–13° C.

5. The physiological properties of the neuromuscular system of *Haliclystus* appear to be intermediate between *Calliactis* and *Cyanea*. There is a demonstrable difference in the response of stalk and calyx which shows emphasis on sequential elicitation by mechanical stimulation of progressively greater intensity of tentacle, local marginal, essentially whole calyx, and finally symmetrical stalk involvement. The significance of this series of events in relation to the normal behavior of the animal is pointed out.

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